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"AN ANALYSIS OF FACTORS HAVING FOLIC-ACID-LIKE ACTIVITY IN AMERICAN DIETS"

Contract number DA-49-193-MD-2299

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3. C. E. Butterworth, Jr., M.D.
Rafael Santini, Ph.D.
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Conventional bacteriological assays for folic acid are non-specific in the sense that they reflect growth to a variety of compounds - eight or more in the case of S. faecalis. We have adapted recent advances in "DEAE" column chromatography to our needs and have performed assays on mixed diets as well as individual foods after preliminary chromatographic isolation of active components. It has been found that almost 90% of the total "activity" is present in forms other than pteroylglutamic acid. After conjugase treatment ordinary American diets were found to contain the following daily amounts of components with folic-acid-like activity:

Component I (unidentified)	- 102 µg
Citrovorum factor (CF)	- 63 µg
Pteroylglutamic acid (PGA)	- 20 µg

Evidence is cited which suggests that certain compounds which possess folic-like activity for micro-organisms may be inactive in human nutrition.

Preliminary evidence indicates that certain vegetables (such as spinach, broccoli, asparagus, lettuce and endive) are poor sources of PGA, although rich in one or both of the other two components. The obvious implication is that if humans have a nutritional requirement for PGA as such, then the vegetables on this list should not constitute the only dietary source of this vitamin.

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THE PTEROYLGLUTAMIC ACID AND CITROVORUM FACTOR CONTENT
OF AMERICAN DIETS AS DETERMINED BY
CHROMATOGRAPHIC ISOLATION*†

by

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Running Page Heading:

The Pteroylglutamic Acid and Citrovorum Factor Content of Foods

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INTRODUCTION

It has not been widely emphasized that folic acid activity, as determined by conventional bacteriological assay procedures, reflects the presence of a number of compounds other than pteroylglutamic acid (PGA). For example, one popular test organism, Streptococcus fecalis, will show a growth response in folic acid free medium to at least ten derivatives of PGA in addition to thymine, hypoxanthine, theobromine, guanidine, uric acid and several other related compounds (1). Another organism, L. casei, shows growth with a somewhat wider range of PGA derivatives including triglutamate forms which do not support growth of S. fecalis (2). Under these circumstances, the practice of expressing the growth supporting ability of crude dietary materials in terms of a single reference standard may not give a true indication of the PGA content of foods, because food may contain a mixture of compounds with folic acid-like activity (3). Nevertheless, most dietary tables on the folic acid content of foods are based on such assays.

The discrepancy between the amount of folic acid activity in diets and the amount of pure PGA required for the treatment of tropical sprue has led to the suggestion that humans may have a specific requirement for PGA (3). It therefore seemed important to assess the quantity of PGA available as pure PGA in foods and to investigate the amount of folic acid-like activity which is present in forms other than pure PGA. A method has been employed in which factors with folic acid-like activity are chemically isolated from foods or mixed diets and individually assayed with S. fecalis.

METHODS

The procedure of the Association of Official Agricultural Chemists (4) with minor modifications was used for assay of folic acid as follows:

The materials and reagents used were:

Assay Medium: The assay medium used was Bacto 318-dehydrated. To rehydrate this basal medium, 75 g were suspended in 1000 ml of distilled water, then heated to boiling for 3 minutes and allowed to cool to room temperature.

Test Organism: Stock cultures of Streptococcus fecalis (ATCC 8043) were prepared by stab inoculation of bacto-micro assay culture agar. Following incubation at 37° C for 18-24 hours, the cultures were stored in a refrigerator at 4° C. An assay inoculum was prepared by subculturing from the stock culture into a tube containing 10 ml of the bacto-micro inoculum broth. This inoculum was incubated at 37° C for 24 hours, then centrifuged under aseptic conditions and the supernatant liquid decanted and discarded. The cells of the inoculum were resuspended in 10 ml of freshly sterilized 0.9% saline solution. This procedure of washing and centrifuging was repeated three times. The washed cells were then suspended in 10 ml of sterile saline solution and one drop of this suspension was used to inoculate the assay culture tubes.

Phosphate Buffer: Prepared as described by Flynn (1).

Standard Folic Acid Solution: Prepared as described by Flynn (4).

Chicken Pancreas (Conjugase) Preparation: Approximately 10 g of fresh chicken pancreas were homogenized in a Waring blender with approximately 35 ml of phosphate buffer at pH 7.2. The homogenate

was then diluted to 50 ml with distilled water. This solution was distributed equally into ten test tubes and stored in the freezer at -20° C. This method is similar to the preparation procedure described by Mims and Laskowski (5). Before use, 1 ml of this solution was diluted to 10 ml with distilled water.

"Free" Folic Acid Activity: Crude diets were homogenized in a one-gallon blender. Water was added to facilitate homogenization. Final total diet weights were recorded to permit calculation of results and aliquots of approximately 250 g were stored in the freezer at -20° C until ready for use.

Duplicate assays for "free" folic acid activity were carried out as follows:

A 5 g portion of the homogenate was placed in a small blender and homogenized for an additional 3 minutes with 35 ml of distilled water and 40 ml of phosphate buffer at pH 7.2. The homogenate was then transferred to an Erlenmeyer flask, along with 100 ml of distilled water to rinse the blender, autoclaved for 15 minutes at 15 pounds pressure and allowed to cool to room temperature. Each sample was then diluted to 200 ml with distilled water and filtered through a Seitz filter pad. Aliquots of the filtrate were taken in duplicate, diluted if necessary to 5 ml with distilled water, added to 5 ml of assay media, autoclaved at 7 pounds for 15 minutes, cooled, and inoculated with one drop of the S. fecalis suspension. After incubation for 18 hours at 37° C, turbidity was determined in a spectrophotometer (Coleman, Junior) at 650 mμ, and compared with a standard PGA growth curve determined simultaneously.

"Total" Folic Acid Activity: Diet samples were prepared as described above for "free" folic acid activity, except that after the initial autoclaving, they were cooled to room temperature and allowed to incubate at 37° C overnight with 5 ml of the diluted chicken conjugase preparation. After incubation the samples were autoclaved 5 minutes at 15 pounds pressure to inactivate the conjugase. The samples were then cooled, diluted to 200 ml with distilled water and filtered through Seitz filter pads. After proper dilution of the filtrate 5 ml aliquots were assayed with 5 ml of the assay media as described in the preceding paragraph. Blank determinations were accomplished by the same procedure on the conjugase preparation without added dietary material.

Citrovorum Factor Activity (CF): In testing for the presence of citrovorum factor (CF, 5-formyl tetrahydropteroylglutamic acid) the organism employed was Pediococcus cerevisiae (ATCC 3081) under conditions similar to those described above except that synthetic CF was used as a reference standard as well as to maintain growth of the organism.

Charcoal Adsorption: After conjugase digestion and Seitz filtration the material in the filtrate was extracted at pH 7.0 with 400 mg of acid washed charcoal (Norit-A, Pfanstiehl) as in the original procedure described for isolation of the norit eluate factor (6). The adsorbed samples were then centrifuged and the supernatant decanted into 500 ml Erlenmeyer flasks. Duplicate aliquots of the supernatant were taken and the amount of folic acid-like substances not adsorbed on the charcoal were determined by the procedure for "total" folic acid activity. The charcoal was then washed with 60%

ethanol. Three fractional elutions of the folic acid-like substances from the charcoal were then carried out using 15 ml of ammoniacal 70% ethanol for each elution. The three eluates were then combined and lyophilized. It was found that up to six extractions of the Seitz filtrate with charcoal yielded a slightly greater quantity of active material, but did not alter the pattern of components on subsequent fractionation.

"DEAE" Preparation and Chromatography: The techniques of "DEAE" (diethylaminoethyl cellulose anion exchange) preparation and for column chromatography were based on those of Toennies and Phillips (7) and of Oliverio (8). A suspension containing 100 g of DEAE and 1 L each of 0.5 M sodium chloride and 0.5 M sodium hydroxide was prepared. This suspension was filtered with suction through filter paper on a sintered glass funnel. The cake was transferred to a beaker, resuspended in 1 L distilled water and refiltered as above. This process was repeated until the wash water reached a pH of 7.0. During the washing procedure the residual suspension of fine particles was decanted and discarded toward the end of each filtration step. Following the water wash, the material was suspended in 1500 ml of 95% ethanol, filtered, and washed finally with 1500 ml of absolute ethanol. The DEAE was then spread out loosely on a glass dish and allowed to dry overnight at room temperature.

Columns were prepared by pouring a distilled water slurry of DEAE into 1 x 30 ml chromatographic columns equipped with a sintered glass

support at the base. The column was packed with air pressure and refilled until a packed column 1 x 25 cm was achieved, leaving about 2 ml of distilled water above the column. Columns may be prepared, stoppered, and stored in a refrigerator or used at once.

The lyophilized eluate sample was placed directly on the top of the column in an amount ranging from 75 to 150 μ g, dissolved in approximately 5 ml of phosphate buffer at pH 7.2. The appropriate quantity of the eluate was calculated on the basis of "total" folic acid-like activity present in the bacteriological assays of the norite-eluates after lyophilization. The material was forced slowly through the prepared column with the aid of air pressure, using care to avoid passage of air into the DEAE. The column was washed similarly with at least 60 ml of triple distilled water.

Elution of the material from the column was accomplished by a concentration gradient system at pH 7.2 containing ascorbic acid as an anti-oxidant. The elution solutions were prepared immediately before use. The upper flask contained 29.25 g sodium chloride, 4.112 g ascorbic acid, 5.72 ml of 4.06 N potassium hydroxide, brought to a volume of 500 ml with 0.5 M phosphate buffer. The lower, or mixing flask, contained 4.112 g of ascorbic acid and 5.72 ml of 4.06 N potassium hydroxide brought to a total volume of 500 ml with 0.5 M phosphate buffer.

Fractions of 3 ml each were collected utilizing a volumetric

fraction collector. Tubes were shielded from light during and after collection. A gravity flow rate of approximately 0.1 ml per minute was used. One ml of each fraction was tested for ability to support growth of S. fecalis or P. cerevisiae as outlined above. The amount of PGA or CF activity present in each fraction was calculated by comparison with a standard growth curve prepared concurrently using pure PGA or CF respectively. Chromatographic curves were drawn by plotting percent transmisssion (turbidity) on the ordinate and tube numbers on the abscissa of arithmetical graph paper.

RESULTS

The "free" and "total" folic acid activity values for 17 separate, usual American diets are shown in Table I. These values compare favorably with those reported for six separate and ordinary Puerto Rican diets which were found to contain a daily average of 65 µg of "free" and 140 µg of "total folic acid activity (9). No growth supporting activity was found in the control determinations on the conjugase preparation so that no correction factor was necessary. Representative menus of the American diets are indicated in the captions of and 2.

Figure 1 illustrates the growth supporting activity of DEAE fractions derived from a diet with and without conjugase treatment. It is seen that most of the growth supporting activity is in the first two peaks and that the activity is increased in each of these after

conjugase digestion. It is also seen that conjugase digestion is associated with the development of a third peak of growth supporting activity. A similar chromatographic pattern consisting of three peaks was observed regularly in 14 diets after conjugase treatment. This suggested that the third peak might be PGA liberated from the naturally occurring polyglutamate forms. Investigation of this possibility by filter paper chromatography and plate bio-assay as described by Nichol, et al., (10), indicated that the material in the third peak has the same R_f value as reference PGA. Additional evidence that the third peak is PGA was the demonstration of a single peak between tubes 40 and 50 when a reference solution of PGA was passed through the DEAE column. There was 95% recovery of the reference material in this peak.

Figure 2 represents a series of experiments which were performed to determine the relationship of the third peak to PGA. The upper curves in each pair represent 0.12 μ g of folic acid activity determined by prior assay. The lower curves in each pair represent the same amount of folic acid activity plus additional synthetic PGA added to each diet sample in amounts of 0.025, 0.030, and 0.025 μ g respectively. Recoveries ranged from 83% to 101% with an average of 95%. Although the position of the peaks varied somewhat according to flow rates, their relative pattern remained the same and the added PGA produced augmentation of only the third peak.

The distribution of folic acid-like activity among the three peaks has been calculated on a percentage basis for ten diet

samples as shown in Table II. Applying these ratios to a mean daily intake of 184 μg of folic acid activity it is found that the three peaks yield 101, 63, and 20 μg , respectively of folic acid activity for S. fecalis in terms of a PGA standard. Assays of folic acid activity in the ten separate diet samples before and after passage through DEAE columns indicated a recovery of 95% ($n = 10$, range = 72 - 109%, mean = 95, standard deviation = $\pm 5\%$).

The diet yielding the lowest total of folic acid activity, i.e., 80 μg , consisted of:

Breakfast: Toast and coffee

Lunch: Bologna sandwich on white bread with lettuce and mayonnaise, 1/2 pint skimmed milk, 1 fresh pear, 5 vanilla wafers.

Supper: Two baked pork chops, mashed sweet potatoes, marshmallows, creamed field peas, 1 slice whole wheat bread, 1/2 pint homogenized milk.

This diet would provide 4 μg of folic acid activity daily as available PGA on the basis of evidence that the third peak is PGA, amounting in this instance to 5% of the total.

Figure 3 illustrates in the shaded area the pattern of growth support for S. fecalis of fractions collected from a DEAE column run of 0.030 μg of reference citrovorum factor (CF, calcium leukovorin, kindly provided by Dr. T. H. Jukes, Lederle Laboratories, Princeton, New Jersey). Support of bacterial growth was observed in only one

narrow zone, i.e., between tubes 20 to 25. The first small peak is due to a clear yellow pigment and does not represent turbidity. The upper curve in this figure is the pattern of growth support for S. fecalis of fractions prepared from a diet containing 0.027 µg of added reference CF. The pattern of the plain diet is shown in Figure 2, curve 2A. It is seen that only the second peak is augmented.

Further evidence that the second peak is CF is presented in Figure 4 in which it may be seen that the same fractions forming the second peak from diets tested with S. fecalis also support growth of P. previasiae. This diet consisted of one pork chop, French fried potatoes, turnip greens, tomato and lettuce salad, mayonnaise, canned peaches, corn bread, butter, homogenized milk. Calculations from these data indicate that the diets assayed in this study yield a daily average of 63 µg of folic acid activity in the form of CF.

DISCUSSION

The need for some form of folic acid in human nutrition has been amply demonstrated by many investigators since 1945, but the minimum daily requirement has not yet been accurately established. Oral doses of 5 to 15 mg daily have been recommended in the treatment of certain so-called folic acid deficiency diseases (11). However, a reappraisal of man's daily folic acid requirement became necessary recently when Sheehy, et al. (12) demonstrated the effectiveness of small oral doses of folic acid in the treatment of tropical sprue. There is evidence that these patients develop and maintain megaloblastic anemia even while consuming a diet which contains an average of 140 µg of folic acid activity

daily according to bacteriological assay (9). Yet the addition of 25 μ g of pure synthetic PGA to the daily oral intake of these patients resulted in the correction of the clinical manifestations of the disease. It is paradoxical that such patients should respond to a dose of folic acid which is smaller than the amount actually present in the diet. This observation serves to emphasize at least one of the limitations of the bacteriological method for assay of crude diets. This observation has also led to the suggestion that man may have a specific requirement for PGA and may be unable to utilize certain PGA derivatives under certain circumstances (3).

It seems unlikely that the beneficial result of the oral administration of 25 μ g of folic acid was merely an additive effect. Several subsequent observations have indicated that the daily requirement for PGA may be less than the total folic-like activity in crude diets as judged by bacteriological assay. Zalusky and Horbert (13) demonstrated a beneficial response in the megaloblastic anemia of scurvy when 50 μ g of PGA were added to a diet devoid of folic acid. Evidence has also been presented through studies of experimental folic acid deficiency in humans (14, 15), that the minimum daily PGA requirement is in the neighborhood of 50 μ g:

Velez, et al. (16) have recently reported the effectiveness of 5 μ g oral doses of PGA in the treatment of the megaloblastic anemia of infancy associated with kwashiorkor. This corresponds to a dose of approximately 1 μ g per kg of body weight, and was given along with a diet which was estimated to contain less than 10 μ g of folic acid

activity per day. Hansen and Weinfeld (17) have reported that 2 μ g per kg of PGA will give a diagnostic reticulocyte response within 10 to 12 days in all cases of megaloblastic anemia in which folic acid deficiency is the only limiting factor.

The present study demonstrates that ordinary American diets contain an average of 52 μ g of "free" folic acid-like activity per day and an average of 185 μ g of "total" folic acid-like activity per day after conjugase digestion. Chromatographic fractionation has shown that the bacteriologically active material present before digestion ("free") is in the form of two factors, neither of which is PGA. Similar fractionation after digestion with chicken pancreas conjugase yields a third component which in two chromatographic systems corresponds to a synthetic reference standard of PGA (Figure 1). This fraction constituted 11% of the total. On this basis it can be calculated that ordinary hospital diets provide an average of 20 μ g of PGA per day (range 4 to 69 μ g). Our observations suggest that persons may consume diets of the type used in this study for prolonged periods of time without developing overt signs of PGA deficiency. Some diets yielded as little as 4 μ g of PGA daily, but it seems reasonable to assume that occasional consumption of liver or other foods would yield greater quantities of the vitamin and thereby prevent depletion of body stores.

The evidence indicates that the second chromatographic peak is CF and that it constitutes 34% of the total folic acid-like activity present in these diets. Chang has reported the presence of CF in a

variety of animal livers as well as in kale and spinach (18).

Bakerman (19) and Silverman, et al. (20), have shown that tetrahydropteroylglutamic acid has growth supporting activity for

P. cerevisiae if protected by ascorbate, and if added aseptically to the culture medium. In the present study the early autoclaving step would tend to eliminate this factor. The fact that the second peak behaves chromatographically like reference CF is a further indication that this component is indeed CF and not another factor which developed CF activity after elution from the column. In terms of its growth supporting ability for S. fecalis CF represents an average of 50 µg daily and is present in greater amounts than PGA.

It is well known that PGA is converted to CF in mammalian liver and that this conversion is blocked by certain folic acid antagonists (21). However, it is not known if PGA alone, or if CF alone will adequately replace other folic acid-active forms in human nutrition. Woodruff, et al., have reported that a 75 µg daily oral dose of CF is effective in treating the megaloblastic anemia of infancy (22). It has been reported that CF is effective in the treatment of tropical sprue (23, 24, 25), but there is disagreement as to its effectiveness in comparison with PGA. Since the latter studies were done using doses of CF which seems large by present day standards, and since the material administered could have contained microgram quantities of PGA, it will be necessary to make further observations on the comparative effectiveness of CF and PGA in human nutrition.

The nature of the first chromatographic peak is not known. It

could be one or more of the several compounds which are known to support growth of S. fecalis under the experimental conditions of this study. Further studies are in progress concerning the nature of this component.

It should be mentioned that the chicken pancreas enzymes used in the present study may not be comparable to human intestinal or pancreatic enzymes. However, this has been a convenient and widely used source of conjugase. It has been reported that intestinal juice obtained from subjects with tropical sprue possesses conjugase activity (9), and such subjects are said to be capable of responding to conjugated form of PGA (26). However, chromatographic separation of folic acid active factors has not yet been carried out on foods after incubation with human intestinal or pancreatic enzymes.

On the basis of its action on synthetic and natural substrates, Kazenko and Laskowski (27) have presented evidence which indicates that the chicken pancreas conjugase should probably be classified as a gamma-glutamic acid carboxypeptidase. It has been suggested that the end product of digestion with this enzyme is the monoacyl-diglutamate, probably due to inhibition by the liberated glutamic acid (27). In the present study the enzyme yielded from mixed diets a product which corresponds to PGA in two chromatographic systems. While it is possible that both the mono- and the di-glutamate forms would have similar elution characteristics, this seems unlikely. The findings suggest that PGA is liberated from the polyglutamate form of diets, and that 20 µg of PGA may be derived daily from average diets. The small quantities of active factors concerned in the present study have precluded chemical

analysis of the number of glutamic acid residues. Work is in progress concerning the folic-acid-active components of individual foods. Chromatographic analysis of liver and certain green vegetables has indicated two or more components as in the present study with mixed diets. This emphasizes the error inherent in methods which express growth supporting activity of a food in terms of only one reference material; a vegetable, for example, may contain most of its activity in a form which is different from PGA. The present study suggests that certain dietary compounds which have folic-acid-like activity for *micro*-organisms may be inactive in the nutrition of patients with tropical sprue.

SUMMARY

1. Folic acid assay by conventional methods using *S. fecalis* has shown that ordinary American diets contain 52 ± 14 μ g of "free" folic acid activity per day. Assays performed after digestion of those same diets with chicken pancreas conjugase yielded 185 ± 67 μ g of "total" folic acid activity per day.
2. Fractionation of the digested material by column chromatography revealed three separate components with folic-acid-like activity for *S. fecalis*. They represented 55%, 34%, and 11% respectively of the total activity.
3. The first peak has not been identified. Evidence is presented that the second peak is citrovorum factor and that the third peak is pteroylglutamic acid.
4. Calculations indicate that these diets may yield under the conditions of this study a daily average of 63 μ g of citrovorum factor and 20 μ g of pteroylglutamic acid.

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5. It is suggested that certain compounds which possess folic-like activity in bacteriological assay systems may be inactive in human nutrition or at least inactive in the nutrition of patients with tropical sprue.

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TABLE I
 "Folic Acid" Activity in Seventeen Average
 American Diets

•
 $\mu\text{g}/24 \text{ hours}$

Free	Total
40	260
73	200
63	260
35	105
58	190
58	189
50	130
53	138
55	220
25	80
33	108
70	185
78	265
78	210
50	120
24	300
41	188
Mean \pm S.D. 52 $\mu\text{g} \pm 14$	184 $\mu\text{g} \pm 67$

TABLE II

Folic Acid-Like Components in Ten Diet

Samples after Elution from DEAE
(Percentages)

Peak I	Peak II	Peak III
49	34	17
38	39	23
5	45	10
60	40	10
42	46	12
52	40	8
58	28	14
88	6	6
50	38	12
68	27	5
Mean \pm S.D.	55 \pm 15	34 \pm 12
		11 \pm 5

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Luncheon 9, Norit Eluates

"DEAE" Fractionation

Curve A - 5 gm. of Diet, Conjugase Treated

Curve B - 20 gm. of Diet, NOT Conjugase Treated

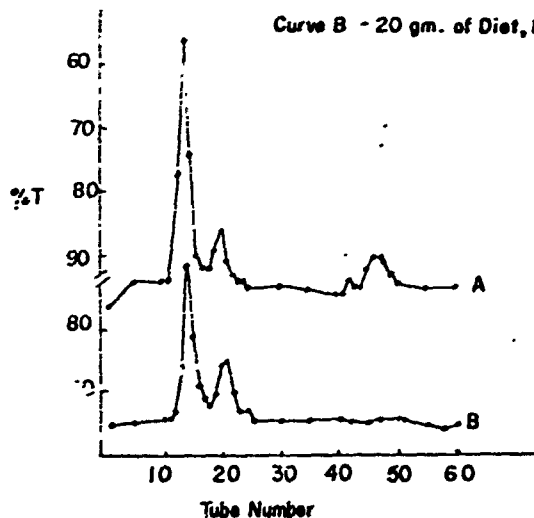
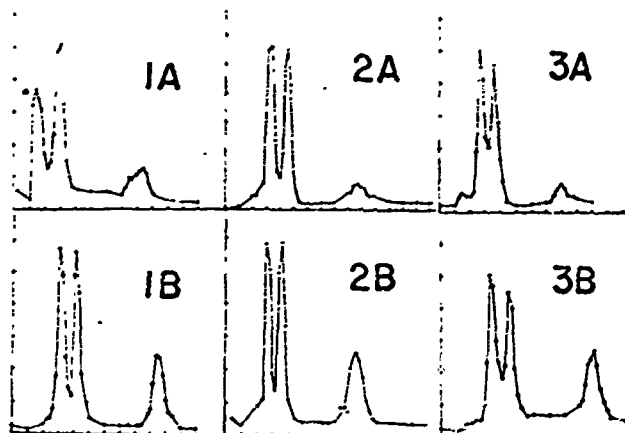


FIGURE 1

The pattern of growth support for S. fecalis of fractions obtained from a diet containing cream of chicken soup, hamburger, onions and gravy, potatoes, squash, asparagus, tomato-lettuce salad, coconut pie, bread, and homogenized milk. The upper curve represents 5 g of dietary homogenate which was incubated overnight with chicken pancreas conjugase. The lower curve represents 20 g of the same diet fractionated without prior conjugase treatment. Although the lower curve represents four times as much dietary material there is no evidence of a peak between tubes 40 and 50 which is the zone of elution for reference PGA.



"DEAE" Column Fractionation of Diets With and Without Added Synthetic PGA

FIGURE 2

The upper chromatogram (A) in each pair represents the pattern of growth support for S. fecalis of fractions obtained from three luncheons. The lower figure (B) represents the results obtained using the same diet containing added PGA and processed in the same fashion, showing accentuation of only the third peak. Menus were as follows:

1A (1305 g) Cream of celery soup, beef stew, broccoli, scalloped potatoes, tossed green salad, cornbread, mocha chiffon pie, homogenized milk;

2A (795 g) Cream of pea soup, ham loaf, potato "boats" with cheese and mustard, cabbage, tomato and lettuce salad, mayonnaise, peaches, tea with lemon and sugar, bread and butter;

3A (1041 g) Cream of pea soup, ham loaf, potato with cheese and mustard, blackeyed peas with snaps, ambrosia salad, butter-scotch pudding, white bread, coffee.

The first peak in 1A appears blunted due to a temporary unequal collection of fractions, but the quantity is the same as in the first peak of 1B.

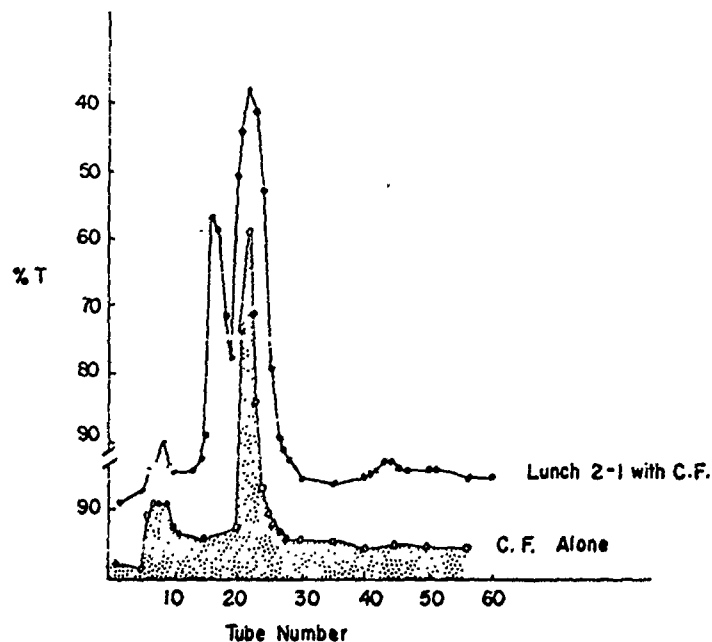


FIGURE 3

The upper curve represents the pattern of growth support for *S. fecalis* of a diet sample containing 0.030 μ g of added CF (calcium leukovorin). The lower (shaded) curve represents the pattern of growth support for the same organism using the same reference material (CF) alone. Comparison of the upper curve in this figure with the curve of the same dietary material chromatographed alone (see Fig. 2, curve 2A) indicates that the addition of CF augmented only the second peak.

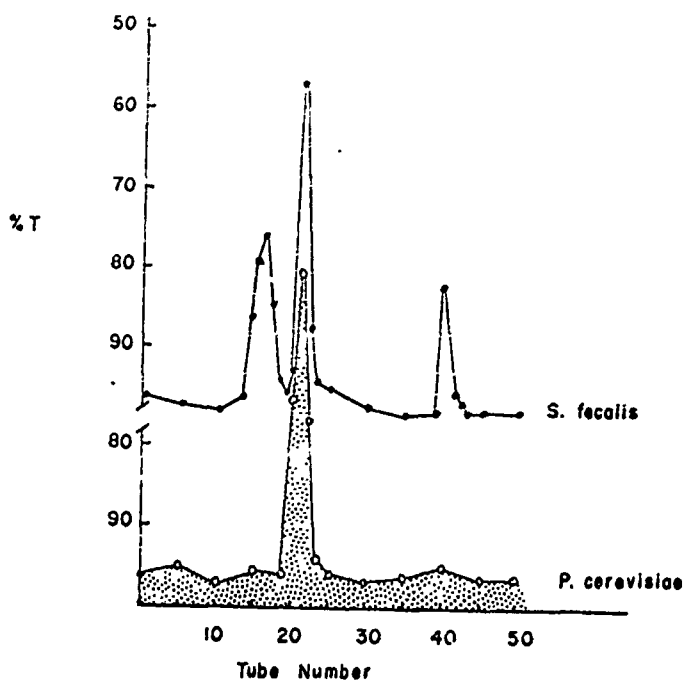


FIGURE 4

The upper curve represents the pattern of growth support for *S. fecalis* of a series of fractions obtained by DEAE fractionation. The lower curve (shaded) represents the same dietary fractions tested for ability to support growth of *P. cerevisiae*, indicating that the second chromatographic component is citrovorum factor (5-formyl, tetrahydro PGA).

ANNUAL PROGRESS REPORT

PART II

The following chromatograms illustrate the fractional analysis of compounds having folic-acid-activity in selected individual foods. On the basis of these findings it has been possible to calculate the amount of folic-acid-like activity which is available in the form of PGA. The results are tabulated below:

	Total FA Activity μg per Gm of Wet Diet or Food	% Activity in Third Peak(PGA)
Spinach	.067	less than 1%
Spinach	.063	less than 1%
Asparagus	.16	less than 1%
Endive	.043	less than 1%
Broccoli	.14	less than 1%
Lettuce	.12	
Yeast	0.40	16%
Yeast	0.50	20%
White beans	1.50	12%
Red kidney beans	1.55	less than 1%
Black eyed peas	1.60	33%
Beef liver	.12	40%
Beef liver	.36	30%
Chicken livers (cooked)	1.0	21%
" " (raw)	1.90	33%
" " (raw)	1.90	25%
" " (cooked)	0.48 (not conjugase treated)	21%

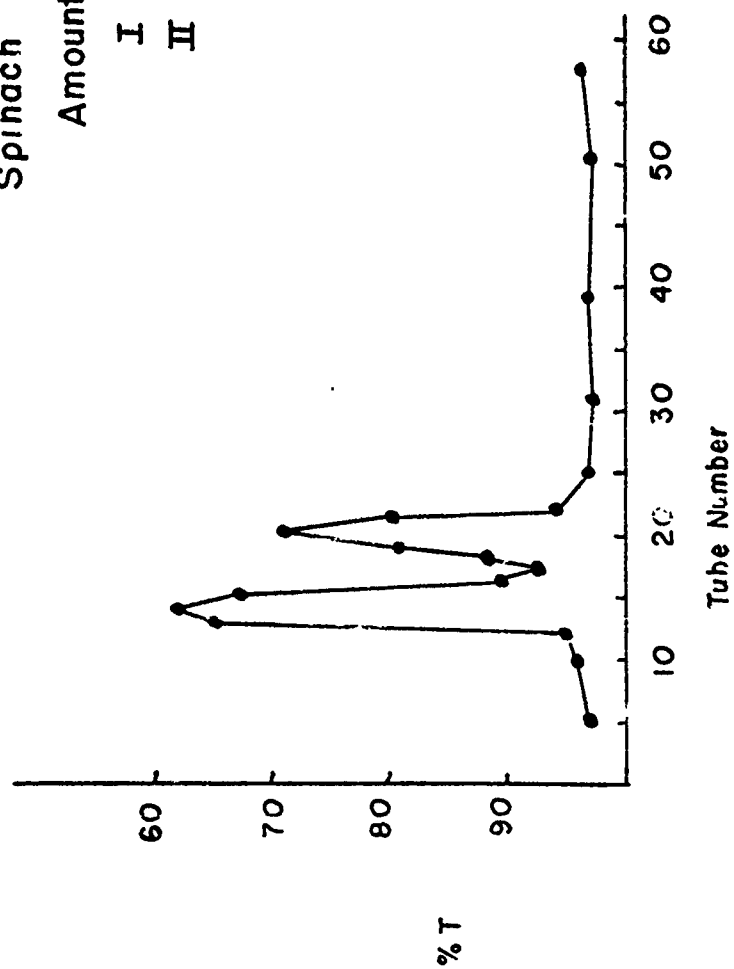
These findings indicate that certain leafy vegetables which have been considered rich in "folic acid" by bacterial assay are poor sources of pteroylglutamic acid as such. In the light of evidence available at present, which suggests that certain persons have a requirement for free PGA, these foods should not be relied upon to provide maintenance amounts of PGA in the diet.

Spinach

Amount passed .06 χ

I	.03
II	.02
	<hr/>
	.05

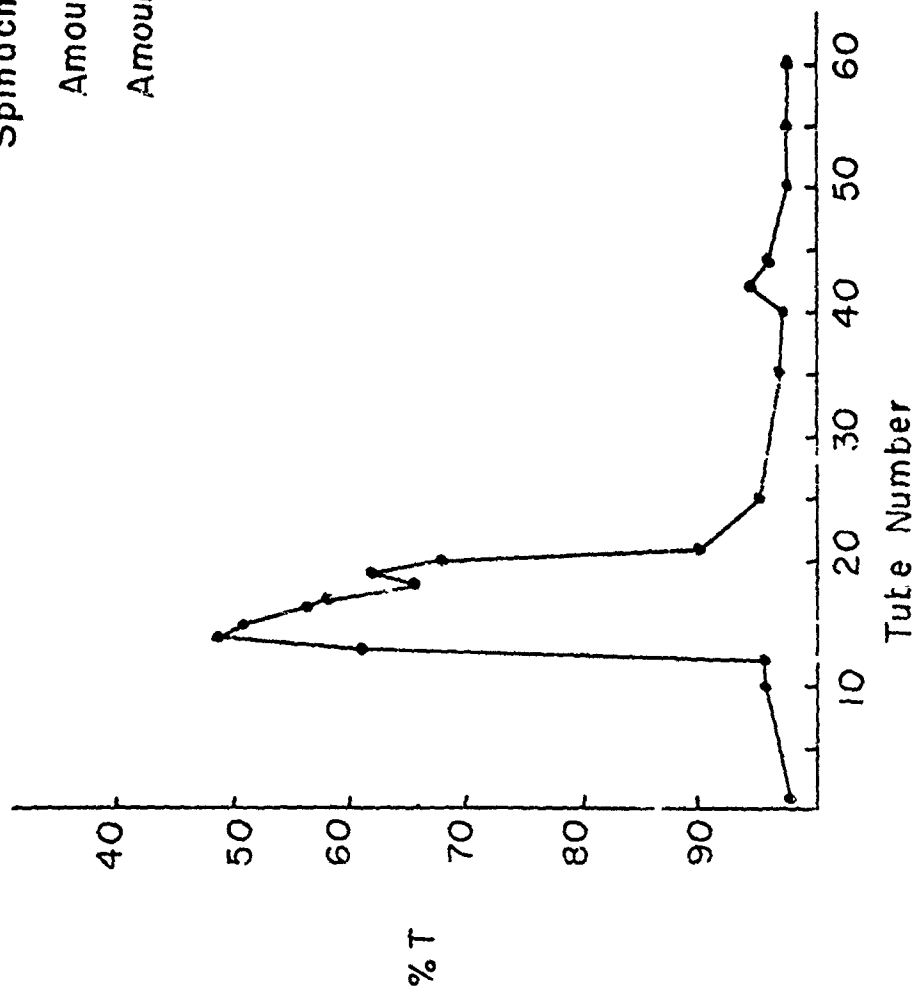
recovered



Spinach 1-8-63

Amount passed .12%

Amount recovered .10%

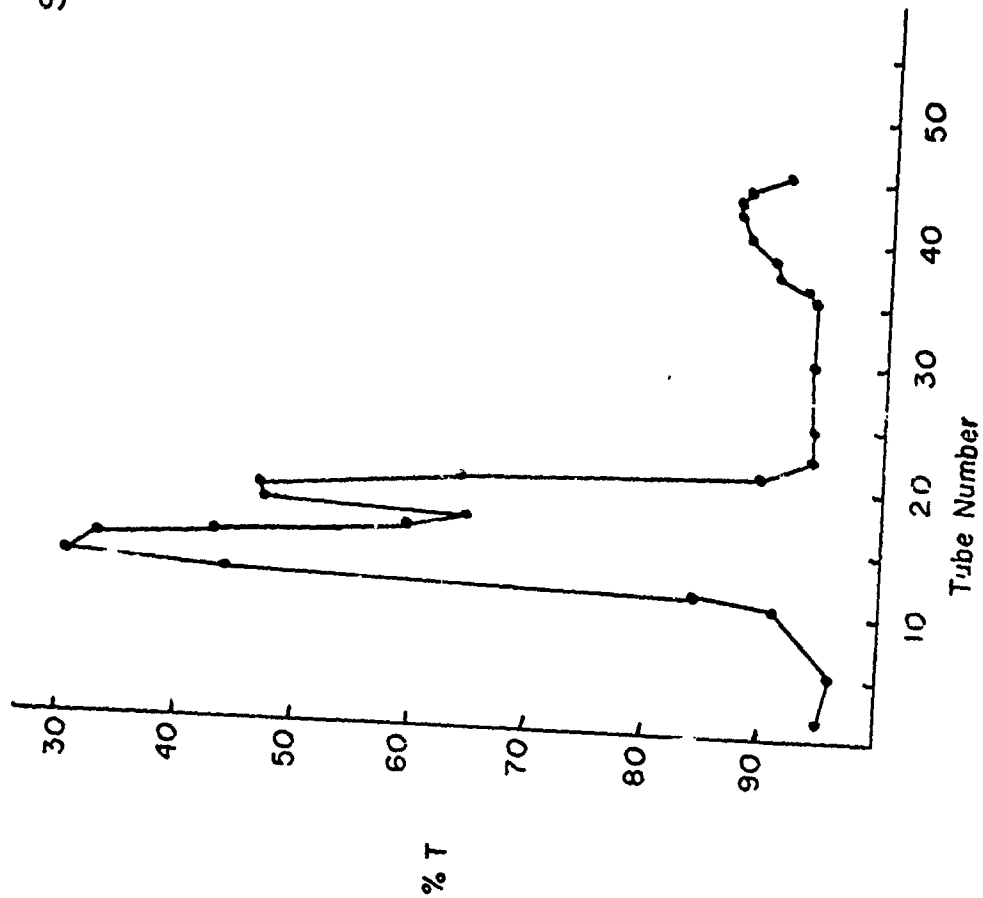


Spinach

1-16-63

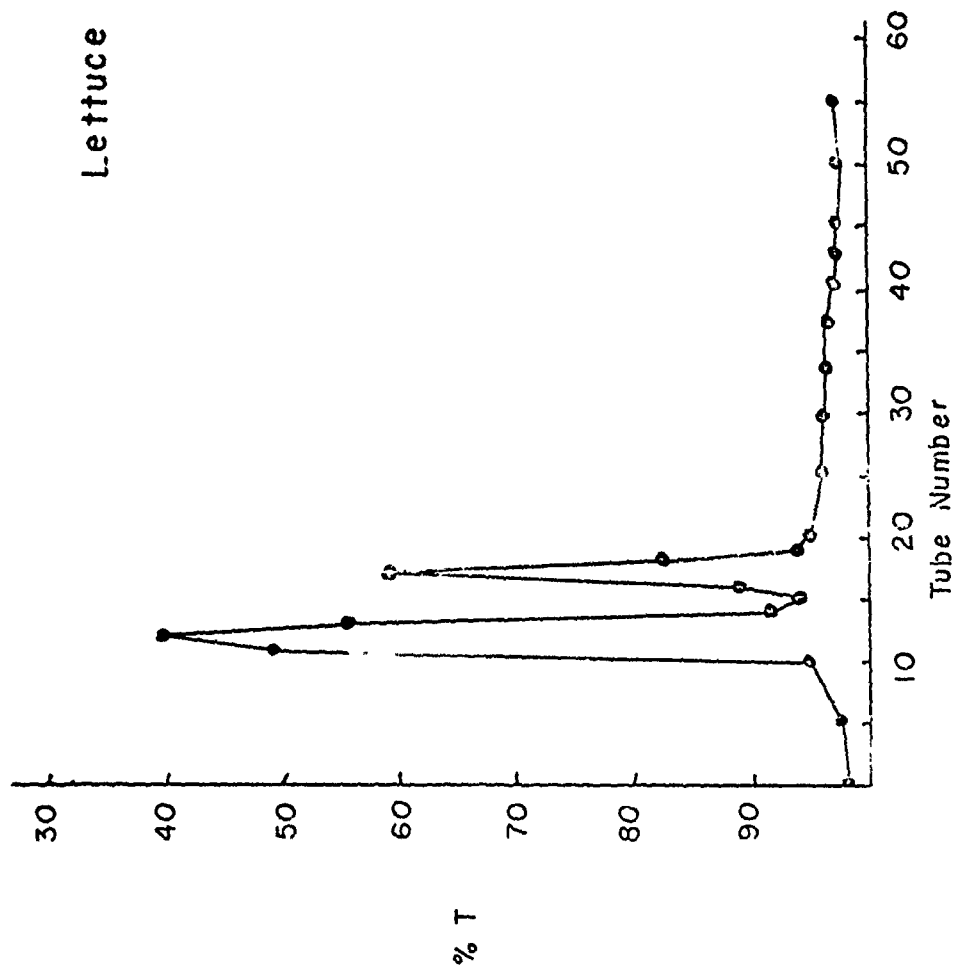
Amount passed .23 χ

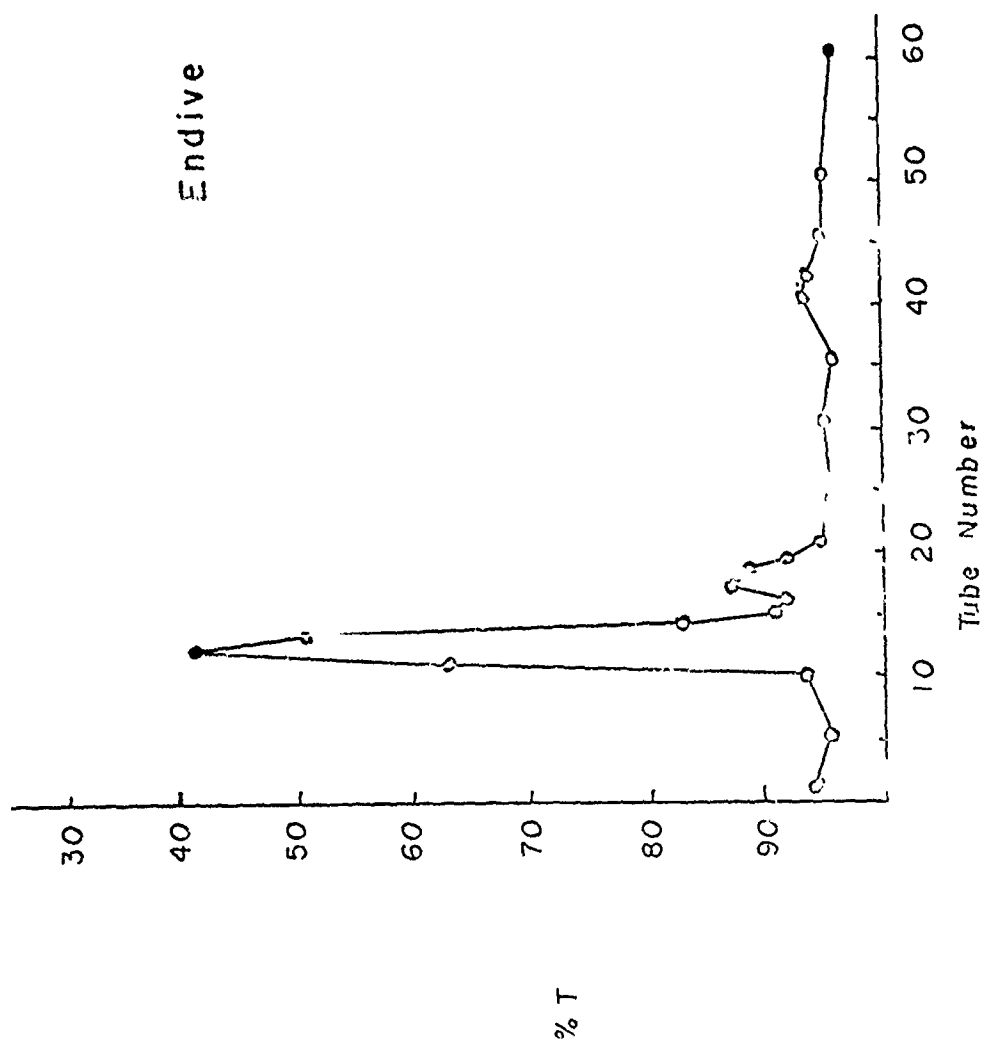
Amount recovered .18 χ



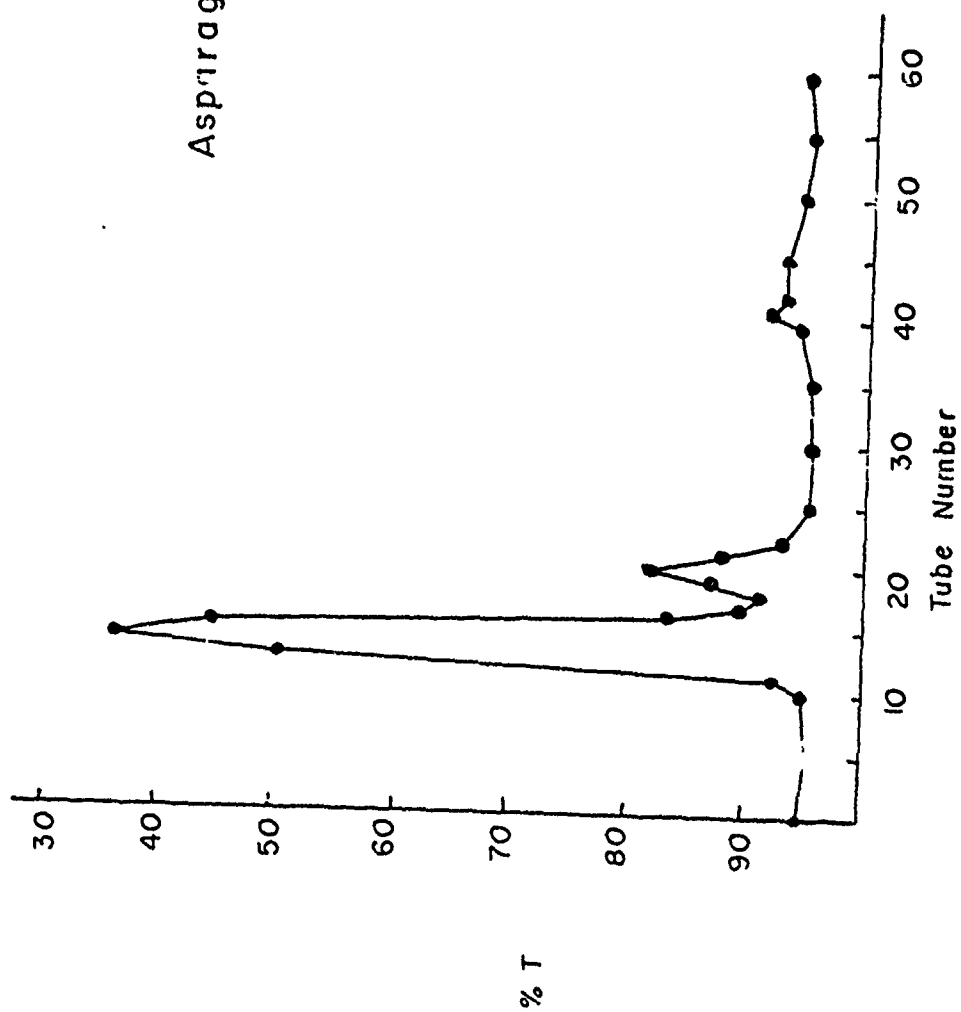
3-20-63

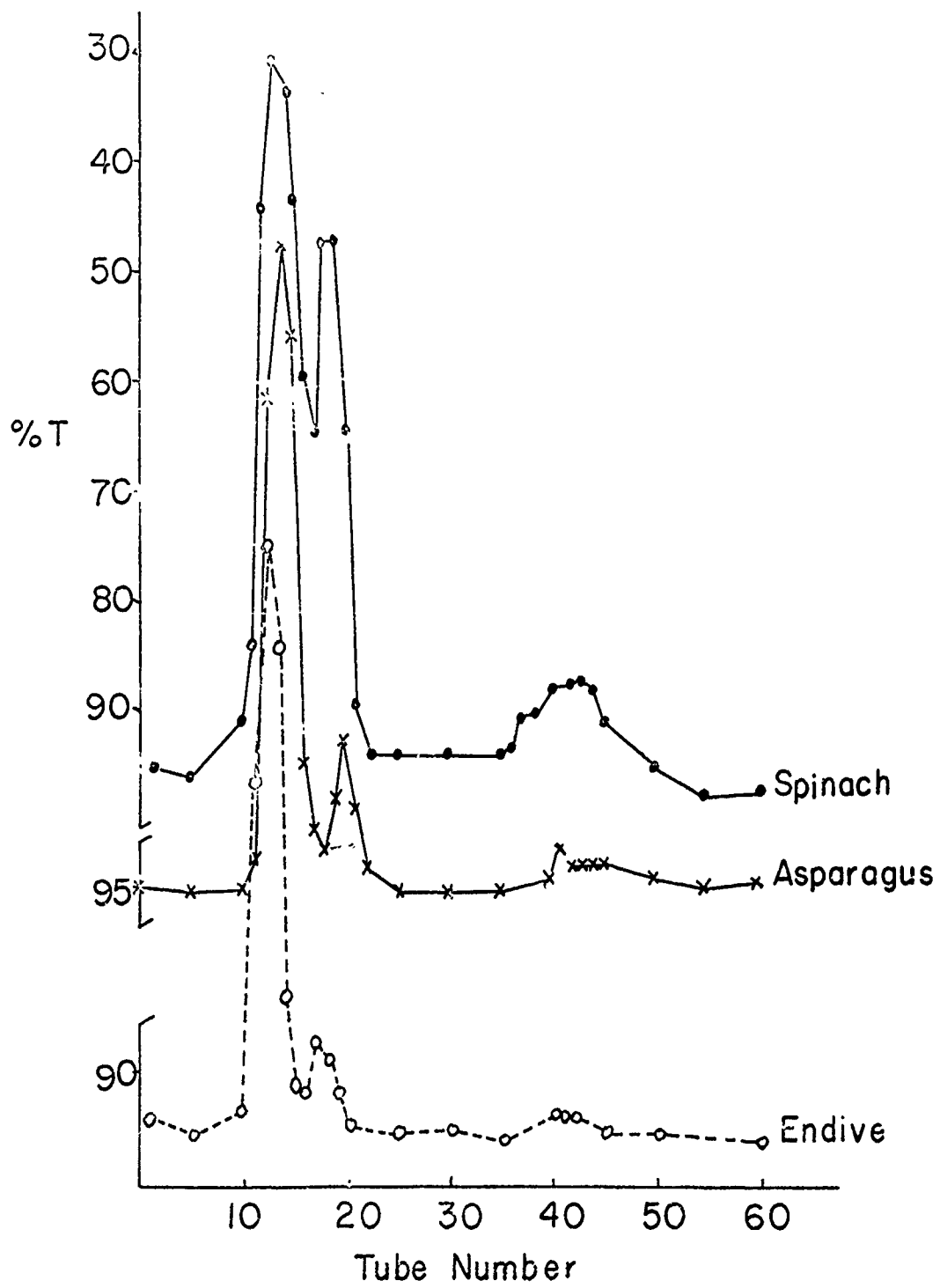
Lettuce



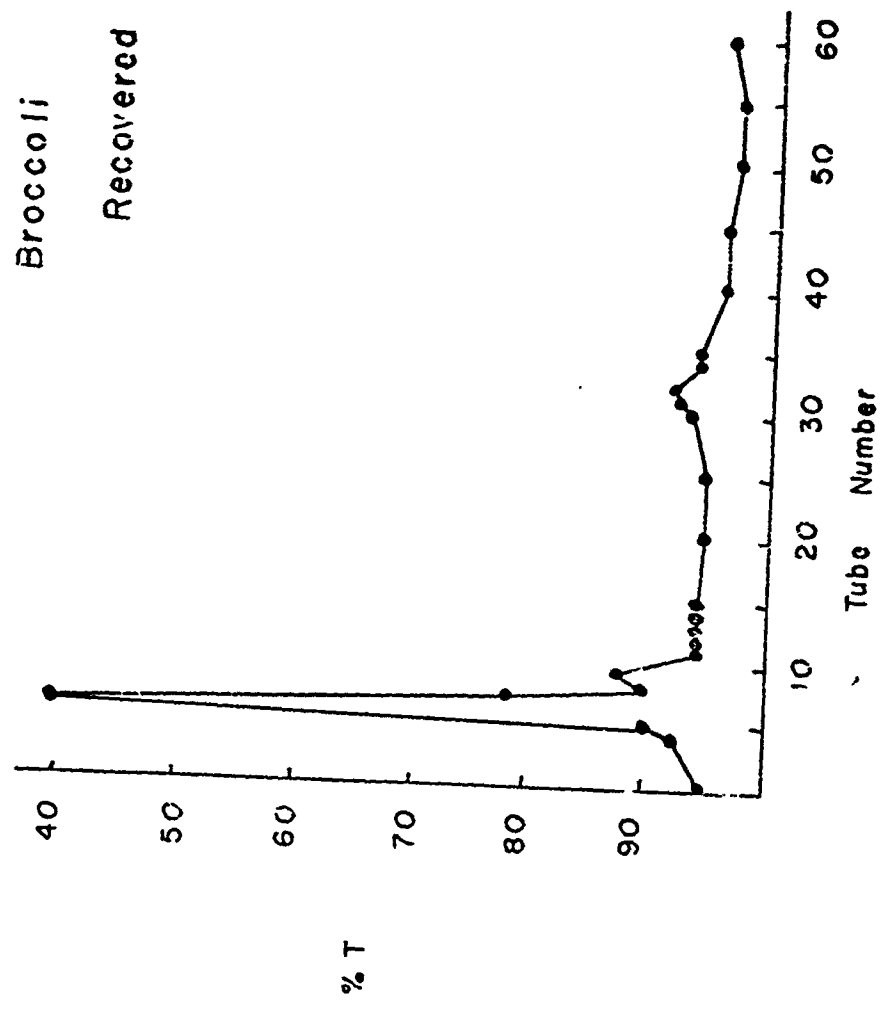


Asparagus





Broccoli .178
 Recovered .1848

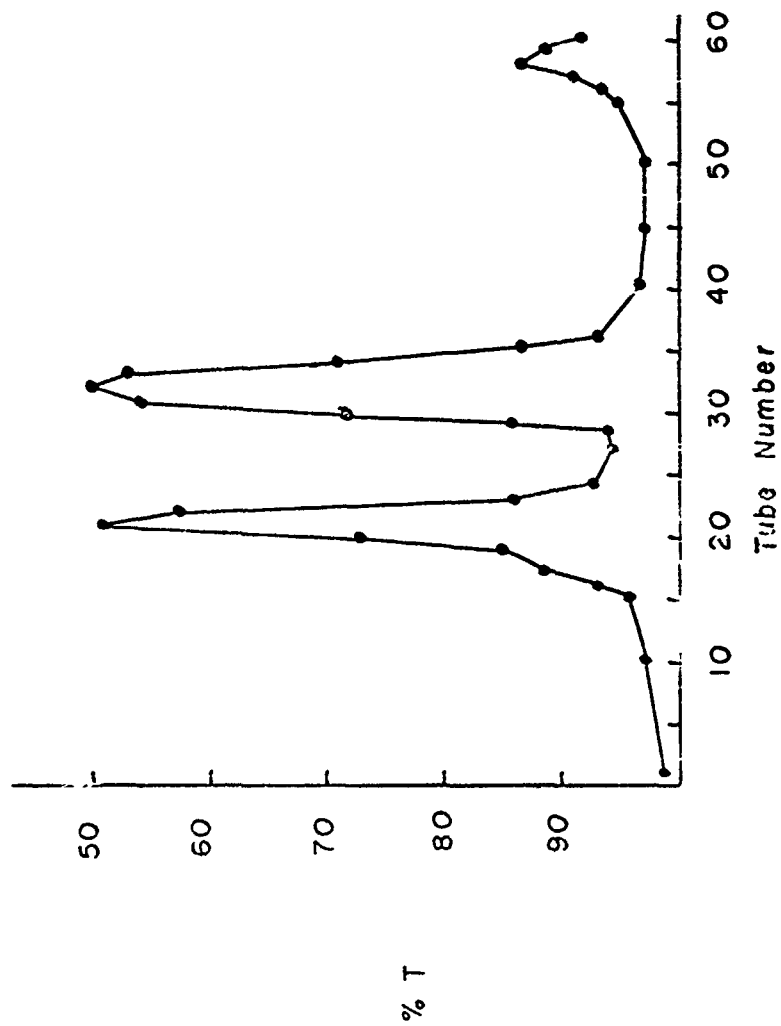


White Beans

Amount passed .218

Amount recovered:

I	.05
II	.064
III	<u>.011</u>
	.125



Red Kidney Beans

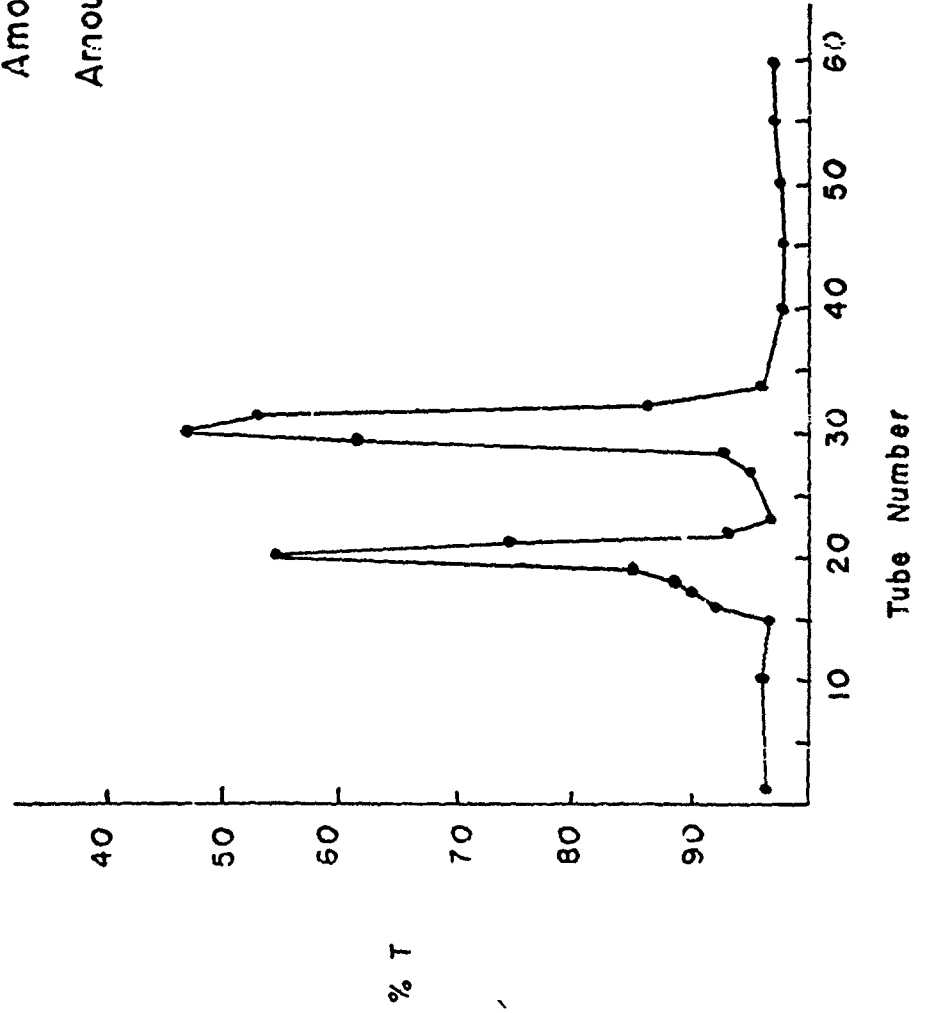
Amount passed .178

Amount found

I .031

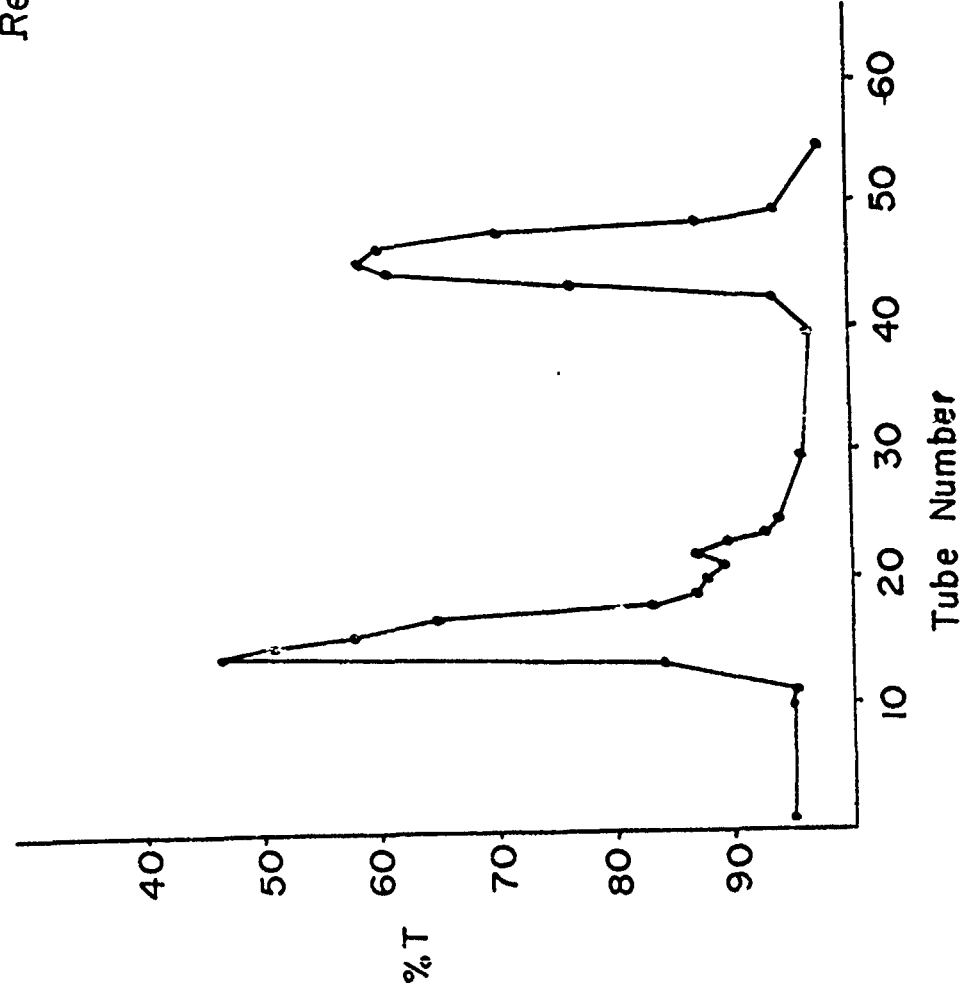
II .044

.075



Chicken Liver

Passed .12 mcgm
Recovered .13 mcgm



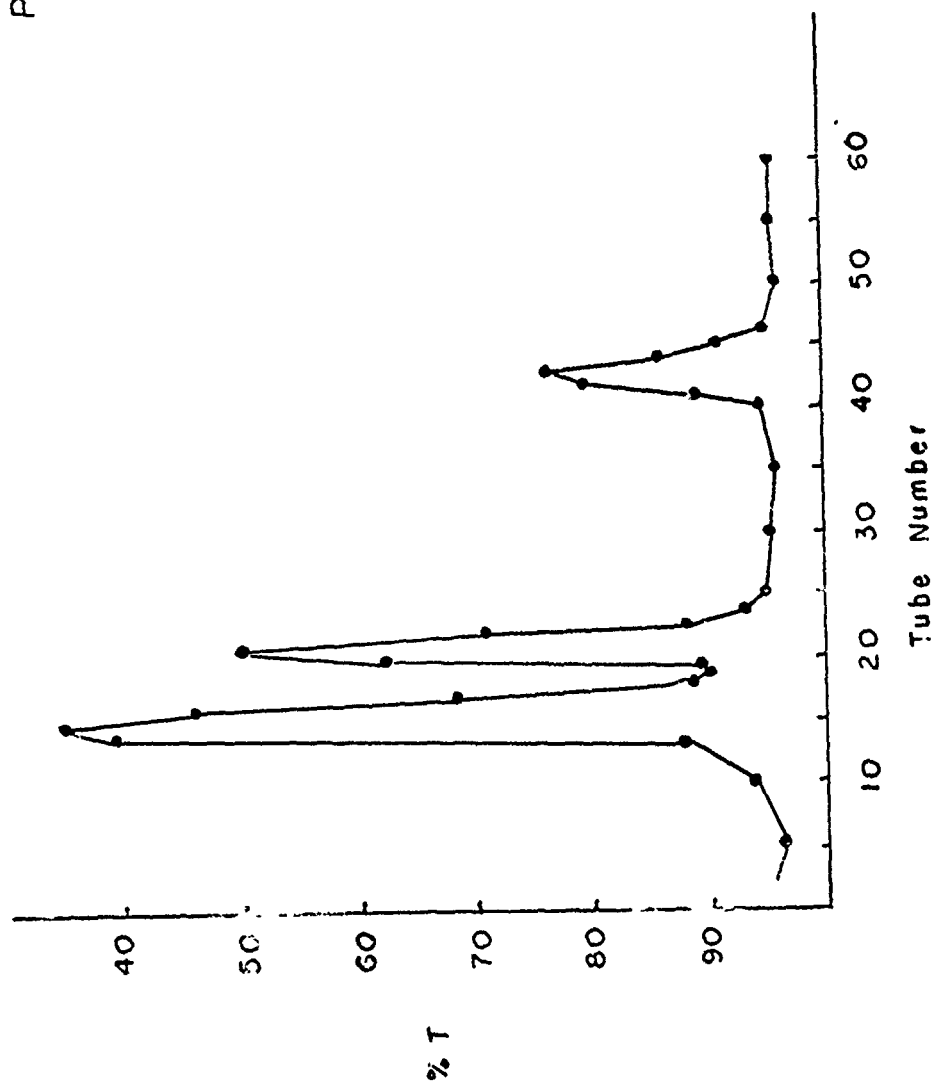
Yeast 'A'

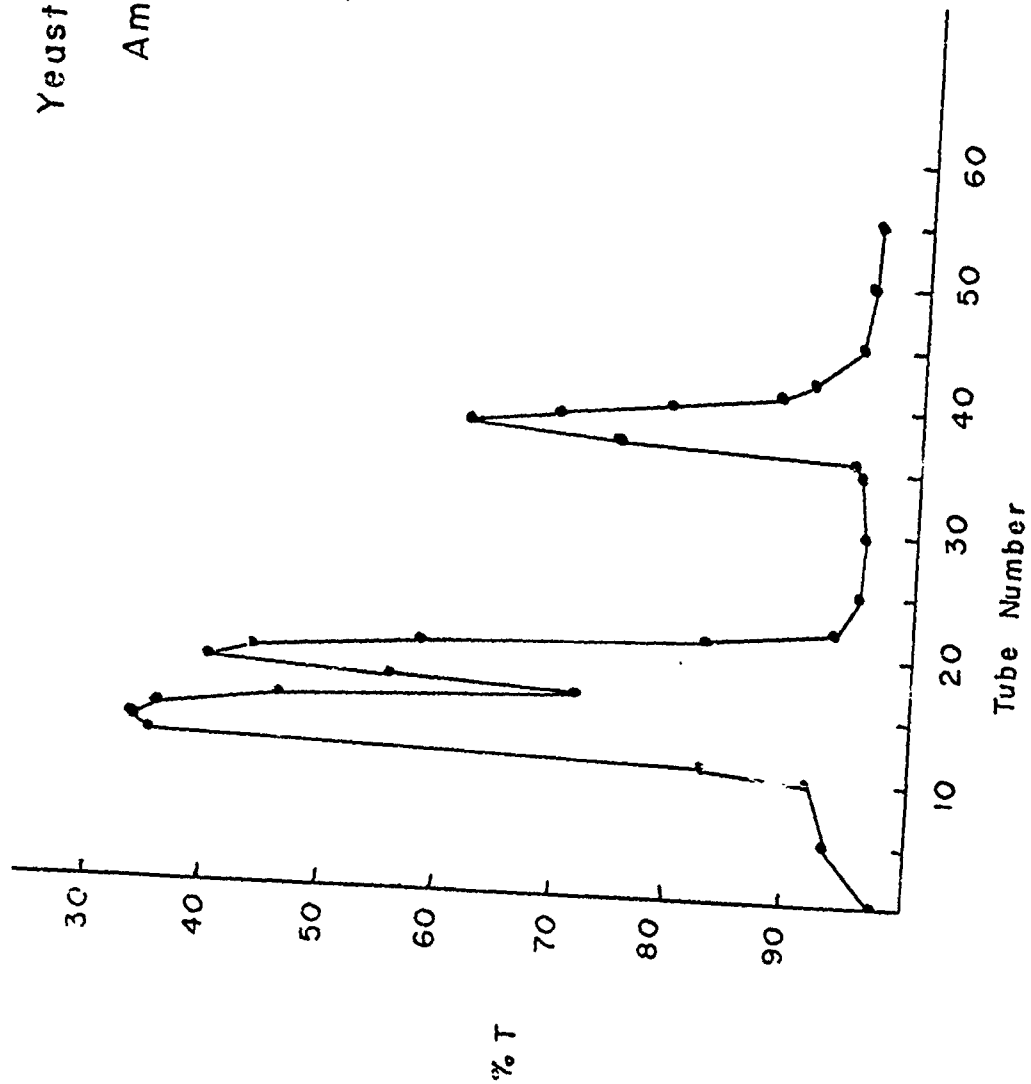
Passed .198

I .059

II .033

III .018
 .110





Yeast B

	Amount	passed	.48
I	.078		
II	.058		
III	<u>.034</u>		
			.170

Black Eyed Peas
Passed .27 γ
Recovered .26

